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PROVISIONAL APPLICATION COVER SHEET

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[ ] ***Additional inventors are being named on the separately numbered sheet attached hereto.***

TITLE OF THE INVENTION (500 characters max)

METHOD FOR TREATING AND PREVENTING AUTISM AND AUTISM SPECTRUM DISORDERS BY USING AUTOTROPHIC AMMONIA OXIDIZING BACTERIA

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METHOD FOR TREATING AND PREVENTING AUTISM AND AUTISM SPECTRUM DISORDERS BY USING AUTOTROPHIC AMMONIA OXIDIZING BACTERIA

Field of Invention

The present invention relates to a composition including ammonia oxidizing bacteria to increase production of nitric oxide and nitric oxide precursors on the surface of a subject and methods of using same to treat and prevent autism and autism spectrum disorders.

Background

Autism is a spectrum of sometimes debilitating development disorders. The "cause" remains obscure, but autism often becomes apparent in the first few years of life. It is during this time that the brain is growing rapidly and forming and reforming many new connections. There is some thought that autism occurs when these connections do not form properly. Among 3 to 4 year olds autistic children, B. F. Sparks et al. show that brain volume was 10 to 13% greater than in normal children and in children with development delays that were not autistic. Brain structural abnormalities in young children with autism spectrum disorder. Neurology 2002 Jul 23;59(2):184-92. Dr. E. H. Aylward, et al. have demonstrated that improper brain growth, and in particular excessive brain volume, has been correlated with autism. Effects of age on brain volume and head circumference in autism. Neurology 2002;59:175–183.

NO is involved in many physiological processes. Because many of the effects of NO are nonlinear and are coupled to many other physiological processes, experimental determination of the effects of NO is not simple, particularly when it is not easy to change basal NO levels. Ragnar Henningsson et al. have indicated that inhibition of NOS with L-NAME can increase NO levels at particular sites. Chronic blockade of NO synthase paradoxically increases islet NO production and modulates islet hormone release. Am J Physiol Endocrinol Metab 279: E95-E107, 2000.

Thayne L. Sweeten et al. has reported that there is an increased level of NO production in autistic individuals. High nitric oxide production in autistic disorder: a possible role for interferon-γ. Biological Psychiatry Volume 55, Issue 4, February

2004, Pages 434-437. Sadik Sogut et al. have also reported higher levels of NO in autistic individuals. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. Clinica Chimica Acta 331 (2003) 111-117. Elevated serum nitrate and nitrite levels are also observed by G. Giovannoni et al. in patients with multiple sclerosis. Raised serum nitrate and nitrite levels in patients with multiple sclerosis. Journal of the Neurological Sciences 145 (1997) 77-81.

One researcher, Lennart Gustafsson has suggested that autism might result from low NO due to inadequate levels of nitric oxide synthase. Neural network theory and recent neuroanatomical findings indicate that inadequate nitric oxide synthase will cause autism. In Pallade V, Howlett RJ, Jain L, editors. Lecture notes in artificial intelligence, Volume 2774, part II. New York: Springer-Verlag, P 1109-14. Gustafsson suggests that the inadequate levels of nitric oxide synthase produces abnormal minicolumn architecture during development, which he suggests might also be produced by low levels of serotonin. Comment on "disruption in the inhibitory architecture of the cell minicolumns" Implications for autism". Neuroscientist 10 (3): 189-191, 2004. January 8, 2004. He suggests that autism might be treated by increasing the activity of nitric oxide synthase in the brain, but offers no suggestions of how to do so. He notes that a nitric oxide explanation provides a rational for some of the seemingly disparate symptoms observed in autism spectrum disorders including comorbidity with epilepsy, motor impairment, sleep problems, aggression, and reduced nociception.

Summary of Invention

The present invention relates to a method of treating an autism spectrum disorder in a subject by positioning ammonia oxidizing bacteria in close proximity to the surface of the subject.

Another embodiment of the present invention relates to an article of clothing for treating an autism spectrum disorder of a subject wherein the article is treated with bacteria adapted to metabolize any of ammonia, ammonium salts, or urea into nitric oxide and/or nitric oxide precursors.

The present invention is also directed to a preparation to be applied to a surface of a subject for treating an autism spectrum disorder in the subject comprising

ammonia oxidizing bacteria adapted to metabolize any of ammonia, ammonium salts, or urea into nitric oxide and/or nitric oxide precursors.

Other advantages, novel features, and objects of the invention will become apparent from the following detailed description of non-limiting embodiments of the invention when considered in conjunction with the accompanying drawings

Detailed Description

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having," "containing," "involving," and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

The present invention relates to a method of treating Autism Spectrum Disorders in a subject by using autotrophic ammonia oxidizing bacteria. The term "treat" is used herein to mean prevent the onset of or retard the onset of a disease or disorder as well as to retard, stop or reverse the progression of disease or disorder after its onset. As used herein, the phrase Autism Spectrum Disorders is defined as is generally recognized, (DSM IV, Diagnostic and statistical manual of mental disorders, 4^{th} ed. Washington, DC: American Psychiatric Association, 1994.) namely Autistic disorder, or Pervasive Development Disorder characterized by severe quantitative deficits in communication, both verbal and non-verbal, social interaction and play, and stereotypical narrow range of interests, Asperger's syndrome, deficient sociability and narrow ranges of interests, and disintegrative disorder, where an otherwise normally developing child severely regresses resulting in severe acquired autism. Examples of Autism Spectrum Disorders include autism, Asperger's syndrome, and Heller's syndrome. Under conventional practice, Autism Spectrum Disorders are limited to fairly severe levels of dysfunction.

Autism is a severe disorder characterized by severe impairment of social interactions. An individual must have multiple and severe deficits to meet the diagnostic criteria for autism. It is to be recognized that many of the attributes of individuals with Autism Spectrum Disorders are observed in other individuals, but to

a lesser degree, a degree that does not reach the threshold for clinical Autism or Autism Spectrum Disorders. Symptoms characteristic of Autism Spectrum Disorders that may or may not reach the diagnostic severity in terms of number and/or degree of Autism Spectrum Disorders are defined herein as autism spectrum symptoms. The severity of those autism spectrum symptoms can also be reduced through the method of this invention. A major use of this invention is to reduce the severity of these autistic symptoms, both in individuals with autism and Autism Spectrum Disorders, and in individuals at risk for developing autism or Autism Spectrum Disorders, and in individuals at risk for developing one or more symptoms of Autism Spectrum Disorders.

According to an embodiment of the invention, nitric oxide, a nitric oxide precursor, and or a nitric oxide releasing compound may be positioned in close proximity to a surface of a subject to treat autism spectrum disorders. The term "treat" is used herein to mean prevent or retard the onset of a disease or disorder as well as to retard or stop the progression of disease or disorder after its onset, or to reduce any symptoms commonly associated with the disorder, even if those symptoms do not reach the threshold for clinical disease.

Any ammonia oxidizing bacteria may be used in the present invention. In a preferred embodiment, the ammonia oxidizing bacteria may have the following characteristics as are readily known in the art: ability to rapidly metabolize ammonia and urea to nitrite and other NO precursors; non pathogenic; non allergenic; non producer of odoriferous compounds; non producer of malodorous compounds; ability to survive and grow in human sweat; ability to survive and grow under conditions of high salt concentration; and ability to survive and grow under conditions of low water activity. Examples of autotrophic ammonia oxidizing bacteria include Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocystis, Nitrosolobus, Nitrosovibrio, and combinations thereof.

Natural bacteria can be used as well as bacteria whose characteristics have been altered through genetic engineering techniques. Bacteria culturing techniques can be used to isolate strains with the above characteristics. A mixture of pure strains would avoid the problems associated with simply culturing bacteria from the skin, which includes the potential growth of pathogens and other bacteria having undesirable characteristics. However, culturing bacteria from the skin and growing them on growth media that simulates the composition of human perspiration may also

be effective at increasing the nitric oxide production rate. A useful method for culturing and isolating such bacteria is to grow them on media containing urea and ammonia plus mineral salts, but without the organic compounds that heterotrophic bacteria utilize, such as sugars and proteins. When isolating autotrophic ammonia and ammonia oxidizing bacteria, it may also be desirable to attempt growth on a heterotrophic media to verify that the autotrophic strain is not contaminated with heterotrophic bacteria. Nitrobacter are inhibited by elevated pH and by free ammonia. In soil this can lead to the accumulation of nitrite in soil which is quite toxic when compared to nitrate. On the skin, addition of an alkaline agent would raise the pH and inhibit the oxidation of nitrite allowing higher concentrations to develop. Thus using an alkaline compound could serve to increase the concentration of nitrite. Talc while being essentially neutral often contains calcium and magnesium carbonates as impurities. Small amounts of these may then make the skin alkaline when dry, but upon sweating the pH would drop and the increased nitrite would be available for conversion to NO. Inhibiting bacteria such as Nitrobacter that reduce the nitrite concentration on the skin is a useful method to further enhance nitric oxide release. Alternatively, Nitrobacter may be included, which will then increase the production of nitrate. Then other bacteria utilizing this nitrate and the other organic compounds on human skin to form nitrite can be used

Bacteria that are useful in this regard are bacteria that metabolize the normal constituents of human perspiration into NO precursors. These include, for example, urea to nitrite, urea to nitrate, nitrate to nitrite, urea to ammonia, nitrite to nitrate, and ammonia to nitrite. In some cases a mixed culture is preferred. The bacteria can conveniently be applied during or after bathing and can be incorporated into various soaps, topical powders, creams, aerosols, gels and salves. One aspect of the invention contemplates application to body parts that perspire the most, such as, for example, hands, feet, genital area, underarm area, neck and scalp. The major difference between these different areas of the skin is the activity of water. The skin of the hands is much drier than that of the feet, normally covered with socks and shoes, due to the increased exposure of the hands to the drying effects of ambient air. It is contemplated that different strains of bacteria may work best on different areas of the body, and a mixed culture of all the types would allow those that grow best to proliferate and acclimate and become the dominant culture present in a specific area. Clothing may also be worn to change the local microclimate to facilitate the growth of

the desired bacteria. For example, wearing a hat may simulate dense hair and help to maintain the scalp in a warmer and moister environment.

Because a normal skin environment is relatively dry, bacteria adapted to low water tension environments are advantageous. One example of a moderately halophilic ammonia oxidizing bacteria is Nitrosococcus mobillis described by Hans-Peter Koops, et al. (Arch. Microbiol. 107, 277-282(1976)). This bacteria has a broad range of growth. For example, while the optimum pH for growth is 7.5, at pH 6.5 it still grows at 33% of its maximal rate. Another more halophilic species, Nitrosococcus halophillus described by H. P. Koops, et al. (arch. Micorbiol. (1990) 154:244-248) was isolated from saturated salt solutions in a natural salt lake. Nitrosococcus oceanus (ATCC 1907) is halophilic but has an optimum salt concentration intermediate between the other two. The optimum NaCl concentrations for the three are 200, 700, and 500 mM NaCl respectively. N. oceanus however utilizes urea and tolerates ammonia concentrations as high as 1100 mM as ammonium chloride. While growth at optimum conditions is the fastest, similar results may be achieved by using more bacteria. Thus while the optimum pH for growth of N. mobillis is 7.5, one can achieve the same nitrite production by using 3 times as many bacteria at pH 6.5. Because the quantities of bacteria in the present invention may be large, a number of orders of magnitude larger than that which occurs within 24 hours of bathing, the fact that the pH of the skin is not optimum for these bacteria is not an inhibition to their use. Because N. halophillus was isolated from a saturated salt solution, it should easily survive the relatively moister human skin environment.

Some bacteria produce nitric oxide directly. One example is described in "Production of nitric oxide in Nitrosomonas europaea by reduction of nitrite", by Armin Remde, et al. (Arch. Microbiol. (1990) 154:187-191). N. europaea as well as Nitrosovibrio were demonstrated to produce nitric oxide directly. Nitrosovibrio is often found growing on rock where the acid generated causes corrosion. It has been suggested by Poth and Focht, "Dinitrogen production from nitrite by a Nitrosomonas isolate." (Appl Environ Microbiol 52:957-959), that this reduction of nitrite to volatile nitric oxide is used as a method for the organism to eliminate the toxic nitrite from the environment where the organism is growing, such as the surface of a rock.

Low basal NO may lead to autism via the mechanism that new connections in the brain are not "well formed", and that this malformation of connections is a result of insufficient basal nitric oxide. Insufficient basal nitric oxide may result from a lack

of sufficient nitric oxide during the formation and/or refinement of neural connections. Formation and/or refinement of neural connections may predominantly occur during sleep.

Additional symptoms exhibited in autistic individuals may also point to low NO as a cause, including increased pitch discrimination, gut disturbances, immune system dysfunction, reduced cerebral blood flow, increased glucose consumption of the brain, increased plasma lactate, attachment disorders, and humming. Each of these symptoms may be attributed to a low basal NO level.

One method to prevent autism is to increase basal NO levels by restoring the previously unrecognized commensal autotrophic ammonia oxidizing bacteria (AAOB) that in the "wild" (under prehistoric conditions) would live on the scalp and external skin and generate nitric oxide from sweat derived urea. I have previously reported that modern bathing practices wash these bacteria off faster than they can proliferate and the loss of the nitric oxide they generate may cause many of the chronic diseases of the modern world, including hypertension, heart disease, obesity, diabetes, and Alzheimer's Disease. NO production on human skin from sweat derived urea by commensal Autotrophic Ammonia Oxidizing Bacteria. Poster P208, Presented at: The 3rd International Conference on the Biology, Chemistry, and Therapeutic Applications of Nitric Oxide / The 4th annual Scientific meeting of the Nitric Oxide Society of Japan May 24-28, 2004.

Increasing basal NO levels through the application of AAOB to the external skin may improve some symptoms found in the autism spectrum of disorders. In common with many other people who are successful in science and technology, I consider that I have a mild form of Asperger's Syndrome. Increasing my basal NO level through application of these bacteria has subjectively improved my ability to think creatively, while decreasing my ability to ignore distracting stimuli.

Autotrophic ammonia oxidizing bacteria are universally present in all soils, where they perform the first step in the process of nitrification, the oxidation of ammonia to nitrite. As obligate autotrophs, they are incapable of growth on any standard media used for isolation of pathogens, and may explain why they have not been identified as human commensals earlier, and may not be pathogenic. All known pathogens are heterotrophic. Many animals instinctively cover themselves with dirt and young children also instinctively play in dirt. It may therefore be nearly impossible for humans living in the "wild" in tropical regions where year round

sweating occurs to not develop a biofilm containing these bacteria on the external skin. Having such a source of NO continuously available over evolutionary time, humans would evolve to utilize that NO in their physiology. It may be that one physiological reason for non-thermoregulatory sweating is to increase NO production on the skin. All mammals have sweat glands and those mammals that do not thermoregulate via sweating (rats, mice, dogs) have sweat glands concentrated on their feet, perhaps to facilitate prevention of infection by heterotrophic bacteria and fungi. Removal of this NO source through modern bathing practices may cause dysfunction.

Axon direction, synaptogenesis in CNS, ANS:

The brain is exquisitely complex and has connections that span many inches. It is well known that neurons are motile, and do move and that axons extend in length, make connections, and retract when misdirected. Inappropriate connections are eliminated and appropriate connections are stabilized. The many connections in the brain are not "random", but are "programmed" in ways that are not fully understood. Various neurotropic factors are implicated in providing chemical cues for the growth cone of the axon to be repelled from and to "home in on." No compound has properties that would allow for purely attractive diffusion over a length of several inches. The time constants for diffusion and axon extension cannot be matched to attainable and detectable concentrations.

Therefore, much of the direction of axons may be repulsive, where axons are repelled from inappropriate brain regions. When the growth cone gets "close enough" it can home in using an attractive diffusant. That these connections span several inches, suggests that multiple neurotropic factors are implicated in the long, medium and short range tropism. The number of neurons exceeds the number of possible neurotropic factors and neurotropic factor receptors. Therefore, many of these factors may be used by more than one neuron. The "effective range" of a potential neurotropic factor depends on its production rate, background concentration, destruction rate and diffusion coefficient. The "ideal" attractive compound would be a small molecule with a high diffusivity, a short lifetime, a low background and low detection limit. NO has such properties. Repulsive compounds could be completely immobile and static and some are likely fixed in the cell membrane. The range of an "attractive" compound must be sufficient to reach the target growth cone, but cannot

exceed the distance over which a growth cone can accurately register a gradient due to diffusion. A repulsive compound may have zero range and need only work on contact. A growth cone must be repelled at many places along its growth path, but may be attracted to only one site where it forms its terminal connection.

The balance between the extension of a growing axon and the length scale which it can retract when misdirected, may determine a length scale in the developing brain. Presumably, one "characteristic length scale" of the brain is the distance between the last repulsive interaction and the final "correct" connection of a growing axon. Presumably, this length scale is on the same order as the range of the attractive diffusant. An axon need not be connected to a specific cell to function properly. Presumably a connection that is "near enough" may allow for subsequent Hebbian refinement to "improve" the functionality of the connection until it was sufficient.

H-J Song et al. have shown that cyclic nucleotides including cGMP cause a change in a neuronal growth cone from repulsion to attraction. Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. Science Vol 281 4 September 1998. cGMP is produced by guanylyl cyclase when stimulated by NO. Thus NO may provide a signal to signal advancing growth cones to home in. The first few axon connections may be made at "random", but once some of the appropriate axons have migrated to the proper region, they may stimulate the release of NO in phase with the action potentials in the migrating axons. "Weak" coupling through NO may be transformed to "strong" coupling via synapse formation. Jeseph A. Gally et al. have suggested that NO is the "second messenger" which links the activities of neurons in a local volume regardless of whether they are connected by synapses. The NO hypothesis: Possible effects of a short-lived, rapidly diffusible signal in the development and function of the nervous system. Proc Natl Acad Sci. USA Vol. 87, 3547-3551, May 1990.

One of the few neural structures where neural growth and connection making can be observed is in chick embryos. The mapping of connections between the retina and the visual cortex of the chick brain goes through significant refinement during development. Nitric oxide has been shown to be essential for this refinement of the topographic precision of the connectivity. During this refinement, NOS is expressed in target areas of the brain and not in the retina. Hope H. Wu et al. have shown that systemic inhibition of NOS prevents the refinement of connectivity. The role of

nitric oxide in development of Topographic precision in the retinotectal projection of chick. J Neurosci. 2001, 21 (12):4318-4325. Yan He has demonstrated that nitric oxide produces axonal retraction while leaving a thin trailing remnant. Microtubule reconfiguration during axonal retraction induced by nitric oxide. J Neurosci. 2002, 22(14):5982-5991. This retraction occurred without large scale depolymerization of microtubules and microfilaments. In the presence of brain-derived neurotrophic factor (BDNF) NO stabilizes neuronal growth cones. Alan F. Ernst et al. stabilized growth cones in contact with BDNF coated beads against NO-induced retraction. Stabilization of growing retinal axons by the combined signaling of nitric oxide and brain-derived neurotrophic factor. J Neurosci 2000, 20(4):1458-1469. Other factors, nerve growth factor (NGF) and neurotrophin-3 (NT-3) did not prevent NO induced growth cone collapse. Hope H. Wu et al. showed that inhibition of NOS increases the number of ipsilaterally projecting ganglion cells by 1000% over controls, yet only 10% of them survived. Involvement of nitric oxide in the elimination of a transient retinotectal projection in development. Science; Sep 9, 1994; 265, 5178. P. Cammpello-Costa et al. showed that blockage of NOS induces increased errors in connectivity and increases lesion-induced plasticity in the rat retinotectal projection. Acute blockade of nitric oxide synthesis induces disorganization and amplifies lesion-induced plasticity in the rat retinotectal projection, J. Neurobiol 44:371-381, 2000.

Marriann Sondell et al. have shown that axon growth is stimulated by VEGF. Vascular Endothelial Growth Factor Has Neurotrophic Activity and Stimulates Axonal Outgrowth, Enhancing Cell Survival and Schwann Cell Proliferation in the Peripheral Nervous System. The Journal of Neuroscience, July 15, 1999, 19(14):5731–5740. VEGF transcription is initiated by HIF-1α, which is initiated by the combined signal of low O_2 and high NO as illustrated by Greg L. Semenza in HIF-1α: mediator of physiological and pathophysiological responses to hypoxia, Invited Review. J. Appl Physiol 88: 1474-1480, 2000; and by Katrin B. Sandau et al. in Accumulation of HIF-1α under the influence of nitric oxide. Blood. 2001;97:1009-1015. Blood flow is known to be strongly correlated with neural activity. Vasodilatation may be mediated through NO activation of guanylyl cyclase and cGMP production leading to relaxation of vascular smooth muscle. Neuronally generated NO may provide the signal to initiate transcription of VEGF and stimulate angiogenesis as well as to couple blood supply with neural activity. With the "sink" for NO being oxygenated hemoglobin, there may be a natural feedback mechanism to

prevent "too much" angiogenesis. The factor that controls brain angiogenesis may be limited to molecules that the blood brain barrier is permeable to, such as NO. K. Kon et al. have shown that inhibition of NOS retards vascular sprouting in angiogenesis. Nitric oxide synthase inhibition by N(G)-nitro-L-arginine methyl ester retards vascular sprouting in angiogenesis. Microvascular research 65 (2003) 2-8. Toshiro Matsunaga et al. have shown that ischemia induced growth of cardiac collateral vessels requires eNOS and NO. Ischemia-induced coronary collateral growth is dependent on vascular endothelial growth factor and nitric oxide. Circulation 2000;102:3098-3103. Dong Ya Zhu has shown that neurogenesis following focal cerebral ischemia requires nitric oxide, and is absent in adult mice lacking the iNOS gene. Dong Ya Zhu et al., Expression of inducible nitric oxide synthase after focal cerebral ischemia stimulates Neurogenesis in the adult rodent dentate gyrus. J. Neurosci. January 1, 2003 23(1):223-229. Presumably, neurogenesis at other times may also require NO. J. D. Robertson et al., have reported that inhibition of nitric oxide synthase blocks tactile and visual learning in the octopus. J. David Robertson, et al,. Nitric oxide is required for tactile learning in Octopus vulgaris. Proc. R. Soc. Lond. B (1994) 256, 269-273; and J. David Robertson et al., Nitric oxide is necessary for visual learning in Octopus vulgaris. Proceedings; Biological Sciences, Vol. 263, No. 1377 (Dec. 22, 1996), 1739-1743.

Many neural connections in the brain are "well formed." Presumably, to achieve this, there may be a mechanism whereby connections can be "tested" and "correct" connections stabilized and "incorrect" connections removed. Presumably, the development of a particular neural structure may involve the proliferation of the relevant cells, projection of axons to the relevant brain volumes, repulsion from inappropriate volumes, connection to the appropriate cells, feedback inhibition of proliferation, followed by pruning of excess or misconnected cells. Presumably the length scale at which these connections can occur depends on the range of the diffusive attractant the migrating axons use to home in on. If that diffusive attractant is NO, anything that lowers the range of NO diffusion may decrease the volume size of brain elements that can be "well connected." A brain which developed under conditions of low basal NO levels may be arranged in smaller volume elements because the reduced effective range of NO.

NO has been implicated as a volume signaling molecule. A unique feature of NO, as a very small hydrophobic molecule is that it can diffuse large distances

compared to other neurotransmitters and pass through lipid membranes and through the blood-brain barrier. The distance which NO can diffuse and achieve a certain terminal concentration depends on the background concentration of NO. The diffusing signal of NO may add to the background NO concentration, and when the sum exceeds the action level, the action of the NO signal may occur. When a signal produces a specific quantity of NO, the range of that signal may depend on the NO background. With a lower background, the quantity of NO required to raise a volume to the action level may be increased. Alternatively, the volume which an NO signal can affect may be reduced when the NO background is lower, or in other words, the effective range of the NO signal may be reduced.

The background concentration dependence on the range of action of NO may explain some effects seen in autism. Some autistic individuals exhibit superior auditory pitch discrimination, reduced auditory "global interference," and/or increased discrimination of "false memories." So called "savant" type abilities are not uncommon. A change in the "homing range" distance for protecting axons may produce improved neural processing of "simple" tasks by increasing local short distance neural connection density in areas providing that "simple" mental function, but it may occur at the expense of more "complex" tasks which require integration of multiple processes over larger volumes through connections spanning longer distances.

Dr. E. H. Aylward et al., has reported that autistic individuals, in their limbic system, have decreased neuron size, increased neuron density, and reduced dendrite complexity. E. H. Aylward, PhD et al., MRI volumes of amygdala and hippocampus in non–mentally retarded autistic adolescents and adults. Neurology 1999;53:2145. Similarly, M. F. Casanova et al, have reported that cells in minicolumns are reduced in size but increased in number. Manuel F. Casanova, et al., Minicolumnar pathology in autism. Neurology 2002;58:428–432. It is also reported by D. G. Amaral et al, that in the amygdala, cells are reduced in size, but increased in number density. D. G. Amaral, M. D. et al., The amygdala and autism: implications from non-human primate studies. Genes, Brain and Behavior (2003) 2: 295–302 Review In fMRI comparisons of autistic and dyslexic brains, similarities have been noted in white matter volume excesses. M. R. Herbert et al. have shown that global volume excesses are observed in autistic individuals, and volume excesses in the parietal lobes are

observed in dyslexics. Martha R. Herbert et al., Localization of white matter volume increase in autism and developmental language disorder. Ann. Neurol 2004; 55:530-540. While some autistic individuals are also dyslexic, rarely autistic individuals are hyperlexic. In one case reported by Peter E. Turkeltaub et al., an autistic boy learned to read before he could speak, and his first spoken word was a word he read. Peter E. Turkeltaub, et. al. . The neural basis of hyperlexia reading: an fMRI case study. Neuron, vol 41, 11-25, January 8, 2004. Autistic individuals showing greater skill in tests such as Block Design have led people, such as H. Tager-Flusbert et al., to propose the weak central coherence hypothesis, that there is inadequate connectivity between different components of the brain, and this inadequate connectivity translates into impaired ability to process gestalts. Helen Tager-Flusberg, et al, Current Directions in Research on Autism. Mental Retardation and Development disabilities Research Reviews 7: 21-29 (2001).

NO may work in concert with NMDA receptors. Excessive NO production inhibits NMDA receptors, which is reported by A. Contestabile to be involved in the feedback control of neuron excitability. Antonio Contestabile, Role of NMDA receptor activity and nitric oxide production in brain development. Brain Research Reviews 32(2000) 476-509. M. Virgili et al report that neonatal blockage of NMDA receptor in rats results in long term down regulation of nNOS. M. Virgili et al., Neuronal nitric oxide synthase is permanently decreased in the cerebellum of rats subjected to chronic neonatal blockade of N-methyl-D-aspartate receptors. Neurosci Lett. 258 (1988) 1-4. R. J. Nelson et al demonstrated that nNOS knock-out mice and mice treated with nNOS inhibitors display excessive aggression toward other mice. R. J. Nelson et al. Behavioral abnormalities in male mice lacking neuronal nitric oxide synthase. Nature 378 (1995) 383-386. NO may therefore be important in neuronal proliferation, neuronal migration, synaptogenesis. Presumably disruption in NO metabolism may have multiple effects in neural development.

Nitric oxide has been demonstrated by B. A. Klyachko et al, to increase the excitability of neurons by increasing the after hyperpolarization through cGMP modification of ion channels. Vitaly A. Klyachko et al., cGMP-mediated facilitation in nerve terminals by enhancement of the spike after hyperpolarization. Neuron, Vol. 31, 1015-1025, September 27, 2001. C. Sandie et al. have shown that inhibition of NOS reduces startle. Carmen Sandi et al., Decreased spontaneous motor activity and startle response in nitric oxide synthase inhibitor-treated rats. European journal of

pharmacology 277 (1995) 89-97. Attention-Deficit Hyperactivity Disorder (ADHD) has been modeled using the spontaneously hypertensive rat (SHR) and the Naples high-excitability (NHE) rat. Both of these models have been shown by Raffaele Aspide et al, to show increased attention deficits during periods of acute NOS inhibition. Raffaele Aspide et al., Non-selective attention and nitric oxide in putative animal models of attention-deficit hyperactivity disorder. Behavioral Brain Research 95 (1998) 123-133.

Inhibition of NOS has also been shown by M. R. Dzoljic to inhibit sleep. M. R. Dzoljic, R. de Vries, R. van Leeuwen. Sleep and nitric oxide: effects of 7-nitro indazole, inhibitor of brain nitric oxide synthase. Brain Research 718 (1996) 145-150. G. Zoccoli has reported that a number of the physiological effects seen during sleep are altered when NOS is inhibited, including rapid eye movement and sleep-wake differences in cerebral circulation. G. Zoccoli, et al., Nitric oxide inhibition abolishes sleep-wake differences in cerebral circulation. Am. J. Physiol. Heart Circ Physiol 280: H2598-2606, 2001. NO donors have been shown by L. Kapas et al. to promote non-REM sleep, however, these increases persisted much longer than the persistence of the NO donor, suggesting perhaps a rebound effect. Levente Kapas et al.. Nitric oxide donors SIN-1 and SNAP promote nonrapid-eye-movement sleep in rats. Brain Research Bullitin, vol 41, No 5, pp. 293-298, 1996. M. Rosaria et al., Central NO facilitates both penile erection and yawning. Maria Rosaria Melis and Antonio Argiolas. Role of central nitric oxide in the control of penile erection and yawning. Prog Neuro-Psychopharmacol & Biol. Phychiat. 1997, vol 21, pp 899-922. P. Tani et al, have reported that insomnia is a frequent finding in adults with Asperger's. Pekka Tani et al., Insomnia is a frequent finding in adults with Asperger's syndrome. BMC Psychiatry 2003, 3:12. Y. Hoshino has also observed sleep disturbances in autistic children. Hoshino Y, Watanabe H, Yashima Y, Kaneko M, Kumashiro H. An investigation on sleep disturbance of autistic children. Folia Psychiatr Neurol Jpn. 1984;38(1):45-51. (abstract) K.A. Schreck et al. has observed that the severity of sleep disturbances correlates with severity of autistic symptoms. Schreck KA, et al., Sleep problems as possible predictors of intensified symptoms of autism. Res Dev Disabil. 2004 Jan-Feb;25(1):57-66. (abstract).

It may be that high NO levels are essential for sleep, and that these high NO levels are also necessary for the neural refinement that may occur during sleep. Night time may be an ideal time to administer large doses of NO to the brain. Basal

metabolism is at its lowest level, therefore, there may be maximum metabolic reserves to compensate for NO induced hypotension and NO induced inhibition of cytochrome oxidase. The individual subject is immobile so the brain need not function to control physical activity. The individual subject is unconscious so the brain need not function to integrate sensory data. It may be that during this night time surge in NO that much of long term potentiation occurs. A large surge in NO may serve to cause misdirected axons to retract, and may strengthen newly formed synapses. The brain activity that occurs during sleep could serve to exercise the newly formed synapses so as to impedance match and optimize the various connections. Using a global mechanism from outside the brain, such as night time sweating on the scalp, may relieve the brain of local regulation of basal nitric oxide level.

It may further be that high levels of NO during sleep may be part of the "normal" "housekeeping" functions of the brain, and may serve in general to refine connections, make short term memory permanent, and "optimize" brain function. It may be that the neural activity that accompanies REM sleep is part of the "testing" of neural connections necessary to "decide" which ones to keep and which ones to ablate. High levels of NO during sleep may be necessary for sleep to be effective for these "housekeeping" functions. It is these high levels of NO generated in part by neural activity of the sleeping brain that may be responsible for the drop in blood pressure observed during sleep. Adrenergic sweating at night, particularly on the scalp, causes the release of urea to the scalp where autotrophic ammonia oxidizing bacteria (AAOB) would generate NO.

S. Ogawa has reported that blood flow in the brain is closely coupled with neural activity, and this close coupling is the basis for fMRI studies where prompt (sub second) alterations in hemoglobin oxygenation (increase in O_2 level) can be correlated with neural activity. Seiji Ogawa, et al., An approach to probe some neural systems interaction by functional MRI at neural time scale down to milliseconds. PNAS September 26, 2000. vol 97 no 19, 10661-10665. In the peripheral circulation, blood flow may be regulated though NO mediated activation of guanylyl cyclase and cGMP mediated relaxation of vascular smooth muscle. Presumably a similar mechanism may hold for the brain vasculature as well. NO generated from neuronal activity may provide NO to relax vascular smooth muscle. However, the promptness of changes in hemoglobin oxygenation might suggest changes in O_2 consumption (by inhibition of cytochrome oxidase by NO) rather than increased supply (though

vasodilatation mediated flow increase). Since mitochondria are regulated by NO, and the operating point of mitochondria is fixed by the instantaneous concentrations of both O2 and NO, any increase in NO may decrease mitochondria activity. Both effects of NO may likely occur simultaneously.

It may also be that measuring NO levels, namely the ratio of NO/O_2 may provide a better measure of the "O_2 diffusive closeness" to O_2Hb, and hence the regulation of capillary spacing in the brain. Presumably, the "O_2 diffusive closeness" of a particular site to oxygenated hemoglobin (O_2Hb) (the source of O_2) must be measured and angiogenesis initiated when it is too low, and capillaries ablated when it is too high. However, it may be that simply measuring the O_2 level is inadequate because the detection of pathologically inadequate perfusion would necessitate pathological O_2 levels. Also, areas with adequate capillary density may not be distinguished from areas with excess capillary density because in both cases O_2 levels are adequate. Measuring NO levels would provide a better measurement. NO has a diffusivity very similar to that of O_2. O_2Hb is the source of O2, and is also the sink for NO, where O_2Hb destroys NO with diffusion limited kinetics. Low NO may therefore be the "signal" that indicates adequate "O_2 diffusive closeness." Low basal NO may lead to the capillary rarefaction observed in many disorders, including hypertension and diabetes. Low basal NO in the brain may lead to capillary rarefaction and hypoperfusion, as well as the characteristic white matter hyperintensity observed in fMRI and which accompanies many neurological disorders. High local levels of NO due to neural activity may signal both the greater innervation of those areas by nearby growing axons, and also greater vascularization through angiogenesis.

Takashi Ohnishi et al. have reported that autistic individuals show decreased blood flow. Takashi Ohnishi et al., Abnormal regional cerebral blood flow in childhood autism. Brain (2000), 123, 1838-1844. J.M. Rumsey et al. have reported that autistic individuals have increased glucose consumption. Rumsey JM, Duara R, Grady C, Rapoport JL, Margolin RA, Rapoport SI, Cutler NR. Brain metabolism in autism. Resting cerebral glucose utilization rates as measured with positron emission tomography. Arch Gen Psychiatry, 1985 May;42(5):448-55 (abstract). D.C. Chugani has reported that autistic individuals have an increased plasma lactate levels. Chugani DC, et al., Evidence of altered energy metabolism in autistic children. Prog Neuropsychopharmacol Biol Psychiatry. 1999 May;23(4):635-41. The occurrence of

these effects may be a result of capillary rarefaction in the brain, which may reduce blood flow and O_2 supply, such that some of the metabolic load of the brain may be produced through glycolysis instead of oxidative phosphorylation. Glycolysis consumes 19 times more glucose than oxidative phosphorylation does to produce the same ATP and produces lactate. While neurons don't produce ATP through glycolysis, other cells in the brain do, namely astrocytes. Capillary rarefaction may both decrease blood flow and increase glucose consumption and increase lactate generation.

It may be that a lack of NO during certain critical periods of development interferes with the formation of high fidelity and efficient neural connectivity over certain length scales. The impairment in connectivity observed in chick visual cortex when basal NO is lowered through NOS inhibition, may also occur in humans when basal NO is reduced by whatever means. Presumably, other neurons use the same NO mediated mechanism that is utilized in the visual cortex. High levels of local connectivity may provide for superior processing of simple neural tasks, at the expense of an inability to integrate those simple tasks into a whole.

Percolation and critical connectivity

Much of the brain is essentially a two dimensional association of individual minicolumns. The main difference between human and animal brains is not the structure of the individual minicolumns, but the greatly increased number and connectivity in humans. Presumably, it is the connectivity of those individual minicolumns that produces the "emergent" human characteristics, such as language, that distinguish humans from animals. If the association of minicolumns is looked at as a connected network, the connectivity of that network may be represented by a length scale. G. Grimmett reported that near the percolation threshold, the overall connectivity of a network becomes very sensitive to small changes in local connectivity. Geoffrey Grimmett. Percolation, Springer-Verlag, 1989. Every element in a functioning neural network cannot be connected to every other element. Neither can every element be disconnected. As the degree of connectivity changes, the degree of connectivity where the properties of the network change most rapidly is at the percolation threshold, where "critical" behavior is observed. That is, various properties of the network diverge at the percolation threshold. For example, slightly below the percolation threshold the length scale of the largest connected cluster is

finite; slightly above the threshold is it infinite. Presumably, the neural network that forms the brain may be above the percolation threshold. Otherwise there would be regions of the brain that are not connected. The brain is not a "simple" network. There are multiple neurotransmitters, perhaps each representing a different network.

It may be that NO, acts as a coupling agent between the various (somewhat) independent networks. "Weak" coupling with NO may facilitate axonal migration and neurogenesis and the formation of "strong" coupling through formation of synapses at the exact "right spot." Some parts of the brain may likely be close to the percolation threshold. There is no strong advantage to a degree of connectivity much higher than the percolation threshold. Connectivity much higher than the percolation threshold is likely to increase the stability of the network, but at the expense of sensitivity of that network to change. Autistic individuals may simply have a slightly too low a degree of local connectivity, which may be brought about by a low basal NO level. Below the percolation threshold, the functionality of a network may be expected to degrade rapidly.

Decreased stability of a neural network would cause increased vulnerability to seizures and it is noted that autistic individuals do have a greater incidence of seizures. Interestingly, I. T. Demchenko et al. have reported that hyperbaric oxygen reduces cerebral NO levels and also induces seizures. Ivan T. Demchenko, et. al., Hyperbaric oxygen reduces cerebral blood flow by inactivating nitric oxide. Nitric oxide: Biology and Chemistry vol 4, No. 6, 597-608 (2000). NOS inhibitors increase the latency to seizure as does L-arginine however, the NO donor S-nitroso-N-acetylpenicilamine (SNAP) significantly shortens it as reported by N. Bitterman. Noemi Bitterman et al. L-Arginine-NO pathway and CNS oxygen toxicity. J Appl Physiol 84 (5): 1633-1638, 1998. NOS does generate NO, however it can also generate superoxide which destroys NO. NOS inhibitors may block both NO and superoxide production. When NO and superoxide are produced together, peroxynitrite is produced. Peroxynitrite may oxidize the Zn-thiolate group in the NOS complex and "uncouple" NOS leading to superoxide formation. Thus the effect of NOS inhibitors on seizure thresholds may be due to its blocking of superoxide formation and not due to blocking of NO formation.

One can look at the brain as a number of somewhat independent processes such as visual processing, auditory processing, individual primitive function generation, language, motor, ANS, etc. Presumably each of these different

"functions" may require an individual brain structure. Presumably that individual brain structure may be a local network with some degree of local connectivity. The percolation threshold for a network may be a critical point. Near the percolation threshold, the properties of the network change exponentially, that is it requires an exponentially smaller and smaller change to effect a macroscopic change in the network the closer to the percolation threshold one is. Presumably different brain structures may require different degrees of connectivity to accomplish the required function. Presumably, for relatively "simple" functions like sensory processing "robust" operation is more important than extreme sensitivity to change. Such structures likely have connectivity well above the critical percolation level. Greater computational effectiveness, such as for functions such as creativity, may require connectivity closer to the percolation threshold. It has been suggested that a "touch" of autism or Asperger's can contribute to intelligence and to creativity. Ed. Uta Frith, Elisabeth Hill. Autism: Mind and Brain. Oxford University Press: 2003, reviewed Nature 428, 1 April 2004, 470-471. A quote attributed to Hans Asperger, "it seems that for success in science or art a dash of autism is essential." Allan Snyder. Autistic genius? Book review: Nature 428, 1 April 2004, 470-471. Perhaps the increased abilities of autistic individuals in some mental areas may be derived from a reduced connectivity in those brain structures leading to a closer approach to the percolation threshold and greater sensitivity to change. A reduced connectivity length is only helpful to a point. Once the percolation threshold is reached, the functionality of the network may rapidly degrade.

If reduced connectivity is the problem in autistic brains, increasing the connectivity may be expected to improve function. If the connectivity is in the near percolation threshold region, the change may be exponential, highly non-linear and improvement may be dramatic.

Impaired ability to "see" gestalts may extend into other areas as well. The inability to perceive "shades of grey", to perceive things as either "black or white", may derive from a lessened ability to integrate numbers of diverse stimuli (or primitive elements) into a whole. Obsessive attachment to specific objects may derive from a similar collapse of the responding brain structures to highly local tiny areas. A significant component of the volume of the brain consists of axons which join different brain regions. Efficient connectivity may minimize path length and minimize axon volume. Inefficient connectivity may result in increased brain volume

without an increase in functionality. The increased brain size observed in autistic children may be a measure of inefficient connectivity.

N. Schweighofer et al. have reported that diffusion of NO can facilitate cerebellar learning. Nicolas Schweighofer et al., Diffusion of nitric oxide can facilitate cerebellar learning: A simulation study. PNAS September 12, 2000, vol 97, no. 19, 10661-10665. This was a simulation study, that showed that plausible NO concentrations and diffusion properties could improve error correcting. M. F. Casanova et al. have reported that there is an increased density of smaller minicolumns in autism. Manuel F. Casanova et al., Minicolumnar pathology in autism. Neurology 2002; 58:428-432. Low NO background may decrease the range at which a NO signal may act, and perhaps provides a rational for the increased density of smaller minicolumns. Just as there may be a signal to initiate neurogenesis, there may also be a signal to stop neural proliferation. NO could provide both signals. A high level of NO close to a source may initiate proliferation, and a low level of NO at the distance where diffusion lowers the NO concentration may terminate it. Tenneti et al. have reported that S-nitrosylation of neural caspase has been shown to inhibit neuronal apoptosis. Lalitha Tenneti et al., Suppression of neuronal apoptosis by S-nitrosylation of caspases. Neuroscience Letters 236 (1997) 139-142. E. Ciani et al., have reported that NO protects neuroblastoma cells from apoptosis due to serum deprivation. Elisabetta Ciani et al., Nitric oxide protects neuroblastoma cells from apoptosis induced by serum deprivation through cAMP-response element-binding progein (CREB) activation. J Bio Chem, 277 (51) 49896-49902, 2002. C. Nucci et al. have reported that NO may be implicated in diverse roles in the lateral geniculate nucleus, from signal transduction to both causing and preventing neuronal apoptosis. C. Nucci et al., Multifaceted roles of nitric oxide in the lateral geniculate nucleus: from visual signal transduction to neuronal apoptosis. Toxicology letters 139 (2003) 163-173.

The brain is not the only place where neuronal connections are made during early childhood. One of the reasons that infants are incontinent is that they lack neuronal control of the voiding functions. Just as the voluntary muscles must be properly innervated to function, so too the various smooth muscles and visceral organs must be connected to the autonomic nervous system (ANS) to function properly. Part of the inability of infants to digest adult foods may derive from a lack of control of the various digestive organs by the ANS. Some of the digestive

disturbances seen with autism may derive from a lack of the proper connectivity of the ANS to the viscera. D. Blottner has implicated Nitric oxide as a messenger in the ANS where nitrinergic pathways are important. Dieter Blottner, Nitric oxide and target-organ control in the autonomic nervous system: Anatomical distribution, spatiotemporal signaling, and neuroeffector maintenance, J Neurosci Res. 58:139-151 (1999). H. Matsuama et al. have reported that vasoactive intestinal protein (VIP) release is regulated by NO. H. Matsuyama Et Al., Peptidergic and Nitrergic Inhibitory Neurotransmissions In The Hamster Jejunum: Regulation Of Vasoactive Intestinal Peptide Release By Nitric Oxide. Neuroscience Vol. 110, No. 4, pp. 779-788, 2002.

D. Blottner has also reported that Nitric oxide is involved in trophic mechanisms in the maintenance and plasticity of the autonomic nervous system. Dieter Blottner. Nitric Oxide and Target-Organ Control in the Autonomic Nervous System: Anatomical Distribution, Spatiotemporal Signaling, and Neuroeffector Maintenance. Journal of Neuroscience Research 58:139–151 (1999) E. Niebergall-Roth et al. reported that release of digestive enzymes by the pancreas is controlled in part by the ANS. E. Niebergall-Roth et al., Central and peripheral neural control of pancreatic exocrine secretion. Journal of physiology and pharmacology 2001, 52, 4, 523-538. H. E. Raybould also reported that release of digestive enzymes is also regulated by compositional feedback from sensors in the gut. Helen E. Raybould. Does your gut taste? Sensory transduction in the gastrointestinal tract, News Physiol. Sci. vol 13, December 1998, 275-280.

Presumably, improper innervation of the gut by the ANS may impair function. T. Wester et al. have shown that the density of neurons in the gut staining positive for NADPH diaphorase (equivalent to NOS) drops markedly in early childhood, and that "nitric oxide is the most important transmitter in non-adrenergic non-cholinergic nerves in the human gastrointestinal tract." T. Wester et al., Notable post natal alterations in the myenteric plexus of normal human bowel. Gut 1999;44:666-674.

Nitric oxide involvement in attachment:

NO is involved in the development of the bonding and smell recognition that occurs in ewes within 2 hour of giving birth. K.M. Kendrick et al., showed that inhibition of nNOS blocks formation of olfactory memory, and this blockage can be reversed by infusion of NO into the olfactory bulb. Kendrick KM et al., Formation of

olfactory memories mediated by nitric oxide, Nature, 1997 Aug 14;388(6643):670-4. J. N. Ferguson et al. reported that oxytocin is essential in the formation of normal social attachment in mice. Jennifer N. Ferguson et al., Oxytocin in the medial amygdale is essential for social recognition in the mouse. Journal Neuroscience, October 15, 2001, 21 (20):8278-8285. G. L. Williams et al. reported that a reduction in oxytocin release following epidural anesthesia in heifers preceded a reduction in maternal bonding type behaviors. G. L. Williams et al., Physiological regulation of maternal behavior in heifers: Roles of genital stimulation, intracerebral oxytocin release and ovarian steroids. Biology of Reproduction 65, 295-300 (2001). G. Gimpl et al. reported that activation of the oxytocin receptor causes activation of nitric oxide synthase. Gerald Gimpl et al., The oxytocin receptor system: structure, function, and regulation. Physiological reviews vol. 81, No. 2, 629-683, April 2001. S. K. Mani et al. reported that inhibition of nitric oxide synthase inhibits lordosis in progesterone stimulated estrogen primed ovariectomized rats. Shailaja K. Mani, et al., Nitric oxide mediates sexual behavior in female rats. Proc Natl Acad Sci, Vol. 91, 6468-6472, July 1994.

W. D. Ratnasooriya et al reported that inhibition of NOS in male rats reduces pre-coital activity, reduces libido, and reduces fertility. W. D. Ratnasooriya et al., Reduction in libido and fertility of male rats by administration of the nitric oxide (NO) synthase inhibitor N-nitro-L-arginine methyl ester. International journal of andrology, 23: 187-191 (2000). R.R. Ventura et al. reported that nitric oxide modulates the activity of oxytocin and vasopressin in the regulation of sodium and water balance. R. R. Ventura, et al., Nitrergic modulation of vasopressin, oxytocin, and atrial natriuretic peptide secretion in response to sodium intake and hypertonic blood volume expansion. Brazilian journal of medical and biological research (2002) 35: 1101-1109. Thus nitric oxide may be involved in pathways known to be important in attachment.

The neurological changes that occur during attachment, either maternal bonding or pair bonding following intercourse can be robust and long lasting, indicating "well formed" connections. C.O. Okere et al. reported that these connections can occur in the space of a few hours, Okere CO, Kaba H. Increased expression of neuronal nitric oxide synthase mRNA in the accessory olfactory bulb during the formation of olfactory recognition memory in mice. Eur J Neurosci. 2000 Dec;12(12):4552-6. The distance over which axons must migrate to form these new

connections may therefore be limited. If the "attachment" neural connections are formed during a period of low NO, perhaps those connections may only be formed in a very local area, thereby forming a powerful "attachment", but perhaps one that may not be modulated by input from other areas. Perhaps this may also lead to dysfunctional attachments, attachment to abusers, attachments to inanimate objects, and perhaps obsessive compulsive behavior.

"Attachment" is in some senses "programmed". Humans (and other animals) are "programmed" to attach to their offspring and to their mates. This characteristic response can occur rapidly (hours in ewes), shorter than the time for neurogenesis, indicating that the behavior originates from neurons that are already present, but that they become connected in different ways during that time.

Immune system interactions

The onset of autistic symptoms in children has been anecdotally associated with childhood vaccinations. While epidemiologic studies have shown no change in incidence in large populations coincident with MMR use or disuse. A consequence of vaccination and activation of the immune system is release of cytokines and induction of iNOS. Elevated plasma nitrate is associated with stimulation of the immune system and is a consequence of iNOS induction. iNOS transcription is mediated through NFκB. M. Colasanti et al. have reported that NFκB is inhibited by NO and so iNOS transcription is inhibited by NO. Marco Colasanti Tiziana Persichini, Marta Menegazzi, Sofia Mariotto, Emanuele Giordano, Claudio M. Caldarera, Valeria Sogos, Giuliana M. Lauro, and Hisanori Suzuki. Induction of nitric oxide synthase mRNA expression suppression by exogenous nitric oxide. J Bio Chem 270, 45, 26731-26733, 1995. Thus a low basal NO level may cause increased iNOS expression and increased NO levels during immune activation (over levels reached with a higher basal NO level). Because iNOS is regulated with a "feed forward" type regulation, if too much iNOS is generated, NO levels may rise to pathological levels, as in septic shock.

iNOS induction may have an effect on neuronal signaling. Increased background of NO may lower the amount on NO necessary to produce effects and may increase the range at which these effects could occur. Effects of NO mediated through nNOS and eNOS would occur at lower thresholds of NO production. Feedback inhibition of nNOS and eNOS transcription may likely occur at lower

nNOS and eNOS expression. U. Forstermann et al. have reported that in vitro following treatment with bacterial lipopolysaccharide (which causes expression of iNOS), that nNOS expression is down regulated. Ulrich Förstermann et al., Expressional control of the 'constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). FASEB J. 12, 773–790 (1998). After the iNOS induced increase in basal NO, basal NO may fall to pre-iNOS levels (or lower). nNOS is synthesized in the cell body, in the endoplasmic reticulum, and is then transported to the site of activity through the axon. This transport necessarily takes some time. Reduced nNOS transcription by high NO levels following immune stimulation during low NO levels may cause NO levels to drop still further. S. H. Fatemi have demonstrated that prenatal viral infection of mice has been demonstrated to produce long term increases and decreases in nNOS expression in different mouse brain regions. Fatemi SH et al., Prenatal viral infection causes alterations in nNOS expression in developing mouse brains, Neuroreport. 2000 May 15;11(7):1493-6 (abstract).

For NO to function as a transmitter between cells, it is necessary that NO be produced at one cell and be detected at another cell. Production of NO by a cell is regulated within that cell and is also regulated by receptors on the surface of the cell. There are very few molecules that diffuse as fast as NO. Feedback regulation of NO production by a cell with a non-NO transmitter, may necessarily entail a significant time lag during which time the NO production would be unregulated and could reach supraphysiological levels.

However, immunizations are not the only sources of immune system activation leading to iNOS induction during early childhood. Early childhood is characterized by many infections, colds, runny noses, diarrheas. While perturbation of NO metabolism might occur as a consequence of any particular immunization, it might equally occur as a consequence of any other immune stimulation. Thus MMR vaccination could be the proximate "cause," for a susceptible individual, but in the absence of MMR, some other immune stimulation, perhaps one of the many diseases of chiildhood, may invariably initiate the change in NO metabolism. Thus the absence of changes in incidence of autism observed in large populations may result from a myriad of other immune system stimulation events of early childhood being equally effective at triggering the autism response in susceptible individuals.

If there is a causal chain between vaccination and autism, a NO mediated pathway may be a conceivable link in that causal chain. However, is it unclear

whiter it is the high levels reached during immune stimulation, and/ or the low level post vaccination that initiates autistic symptoms. Low levels post iNOS stimulation likely initiate autistic symptoms. Development does not occur all at once, but it is an ongoing process. Any disturbance to that process may likely be ongoing as well. In the absence of AAOB generated NO, basal NO levels may become unstable. Low NO leads to increased iNOS expression during immune stimulation and a drop in eNOS and nNOS leading to still lower basal NO levels. Thus, each instance of immune stimulation could cause the basal NO level to ratchet lower. In the "wild"chronic infection with parasites or colonization of the skin with AAOB may exert a stabilizing effect on basal NO levels. The desire of individuals in developed regions to remain free from parasites may increase susceptibility to other disorders. Similarly, a biofilm of AAOB may raise basal NO levels and exert a stabilizing effect on NO levels.

Dr. N. A. Halsey et al. reported that an immune system deviation has been observed in autistic children, characterized by a decrease in Th1 cells and an increase in Th2 cells. Neal A. Halsey et al., Measles-Mumps-Rubella Vaccine and Autistic Spectrum Disorder: Report From the New Challenges in Childhood Immunizations Conference Convened in Oak Brook, Illinois, June 12–13, 2000. Pediatrics 2001;107(5).URL:http://www.pediatrics.org/cgi/content/full/107/5/e84. R. C van der Veen et al noted that Th1 cells, when incubated with antigen, generate NO which inhibits T cell proliferation. Roel C. van der Veen, et al., Antigen Presentation to Th1 but Not Th2 Cells by Macrophages Results in Nitric Oxide Production and Inhibition of T Cell Proliferation: Interferon-γ is essential but insufficient, Cellular Immunology 206, 125–135 (2000) doi:10.1006/cimm.2000.1741, available online at http://www.idealibrary.com. C. S. Benn et al reported that immune system deviation has been seen to increase with increased number of serious infections in early childhood. Christine Stabell Benn, Mads Melbye, Jan Wohlfahrt, Bengt Bjorksten, Peter Aaby. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life, BMJ, doi:10.1136/bmj.38069.512245.FE (published 30 April 2004). Thus a "NO ratchet" in children may lead to a progressively worse immune deviation.

Nitric oxide metabolism:

Nitric oxide is produced in the gut by reduction of dietary and salivary nitrate by heterotrophic bacteria. This reduction occurs in two steps, first to nitrite by nitrate reductase and then to nitric oxide by nitrite reductase. Milk contains abundant xanthine oxidoreductase which can also catalyze the reduction of nitrate and nitrite to NO as reported by Ben L. J. Godber, et al. in Reduction of Nitrite to Nitric Oxide Catalyzed by Xanthine Oxidoreductase. The Journal Of Biological Chemistry, Vol. 275, No. 11, Issue of March 17, pp. 7757–7763, 2000 Excessive NO from this route can cause "blue baby" syndrome which results from oxidation of blood hemoglobin to methemoglobin. Methemoglobin is not toxic, however it does not carry oxygen and in excessive quantities can cause hypoxia. T. Ljung et al showed that nitric oxide is produced in the gut by children with active inflammatory bowel disease, where rectal NO was increased approximately 100 fold over that of healthy children. Tryggve Ljung et al., Increased rectal nitric oxide in children with active inflammatory bowel disease, J Pediatric Gastroenterology and Nutrition, 34:302-306, 2002. Fecal NO was not increased over that of healthy children, implicating a source other than bacterially generated NO (however, as their assay method appeared to be aerobic, it may not have detected the anaerobic NO production expected from bacterial nitrite reductase). An increased NO observed during inflammatory bowel disease may be an adaptive reaction to low basal NO levels.

E. Weitzberg et al have reported that humming increases NO production in the nasal passages. Eddie Weitzberg et al., Humming greatly increases nasal nitric oxide, Am J Resp Crit Care Medicine Vol 166. 144-145 (2002).The NO production is limited by diffusion of oxygen to the active enzyme. Humming increases the gas exchange and so increases NO production and NO measured in nasal air. The NO in the air is inhaled, but most of it would be oxidized to nitrate in the lung. However, the concentration of NO at the site of generation is higher, and some may diffuse into the blood supplying the nasal passage, which drains into the various sinuses in the brain. Humming, which is an observed characteristic behavior of some autistic individuals, may increase NO levels.

R. Henningsson et al have shown that chronic inhibition of NOS with L-NAME in mice unexpectedly increases total pancreatic islet NO production. Ragnar Henningsson et al., Chronic blockade of NO synthase paradoxically increases islet NO production and modulates islet hormone release, Am J Physiol Endocrinol Metab 279: E95-E107, 2000. However, the regulation of NO synthesis is exceedingly

complex. Of all the normal metabolic products, NO is one that inhibits respiration. Sufficiently high NO levels will shut down respiration and can cause cell damage. NO is part of the mechanism by which foreign cells are killed, so immune cells may have the capacity to generate cytotoxic levels of NO. Cytotoxic levels of NO cannot be regulated at the source of NO because cells there are killed. Therefore, the regulation may be separated in time or space from the site of NO generation. Inducible NOS may separate the regulation of high NO production in time. Separation in space may require a different (as yet unknown) messenger molecule.

NO is produced in response to activation of many different receptors. For example, K. Chanbliss has shown that an estrogen receptor causes the release of NO, Ken L. Chambliss et al., Estrogen modulation of endothelial nitric oxide synthase. Endocrine reviews 23(5):665-686. P. Forte has demonstrated that women are observed to have higher levels of NO metabolites, and reduced incidence of diseases associated with low nitric oxide, including hypertension and cardiovascular disease Pablo Forte et al., Evidence for a difference in nitric oxide biosynthesis between healthy women and men. Hypertension, 1998;32:730-734. The different incidence of autism between males and females may derive from an increased basal NO level in females due to increased estrogen mediated NO release.

Nitric oxide and stress

NO tonally inhibits cytochrome oxidase by competitive inhibition with O_2. This inhibition has important physiological effects, in that the delivery of O_2 to individual mitochondria is by purely passive diffusion. Were there no regulation of O_2 consumption, the mitochondria closest to the O_2 source may consume the most O_2, and mitochondria farther away may get less or none. Competitive inhibition with NO, may allow the metabolic load to be distributed over many mitochondria. This may be important in tissues where the O_2 consumption is highly variable, such as in muscle. The O_2 consumption of heart muscle can vary by nearly an order of magnitude. Because O_2 delivery is by passive diffusion, and the geometry of the source and sink doesn't change (there is some increased vascular recruitment, but not an order of magnitude) and the O_2 source (partial pressure of O_2 in the vasculature) doesn't change much, that when the O_2 flux changes by an order of magnitude, the O_2 gradient may change to produce the increased driving force for O_2 diffusion. The O_2 concentration at the mitochondria under conditions of high O_2 consumption may be

less in order for more O_2 to diffuse there. To increase the O_2 flux an order of magnitude at constant source and geometry, the O_2 sink concentration may drop an order of magnitude. If the O_2 consumption increases an order of magnitude while the concentration drops an order of magnitude, the enzyme activity may increase two orders of magnitude. In order to increase metabolic capacity, NO levels may be reduced. This is the "feature" of superoxide production during hypoxia. Superoxide destroys NO and so disinhibits the mitochondria O_2 consumption, allowing mitochondria to consume O_2 even at very low O_2 concentrations. The very low O_2 concentration may allow O_2 to diffuse to where it is being consumed. Superoxide is undesirable, because it damages proteins. However, not enough ATP is worse because then the cell doesn't have the capacity to respond and will necrose.

Nitric oxide regulation and feedback:

NO is generated at diverse sites and then diffuses to diverse other sites where the action of NO is exerted through diverse mechanisms. While NO is a rapidly diffusing gas, and has a "short" diffusion path length, each site may integrate the total NO signal that it receives. A reduction in the basal nitric oxide level may reduce the background level of NO. A reduced background level of NO may result in a decrease in the effective range of NO produced as a second messenger. With a lower background level, the transient NO source may activate a downstream target, may be more diluted and so may have a shorter range at which it reached activating concentrations. It is this shorter range of action that may be important in the malformation of neural connections. The migrating axons may not get "close enough" to receive the NO signal that they need to "home in" on. Axons that do get "close enough" do make good high density local connections, and may perhaps be the explanation for increased aural discrimination.

When an NO source is part of a feedback loop, that source may then be regulated to produce higher levels of NO, which may compensate for the lower background level. The concentration at the NO source to achieve the regulated level after diffusing to the NO sensor may be higher, and may be much higher than with a higher background level. Cells closer to the source than the NO sensor may then be exposed to higher NO levels than "normal." Cells farther away from the source than the NO sensor may be exposed to lower NO levels.

Virtually all important metabolic systems are under some type of feedback control. Nitric oxide may be involved in many feedback control loops, including the regulation of peripheral vascular resistance by shear stress dependant NO release followed by vessel dilatation. A difficulty with the feedback control of NO is that NO diffuses readily, and it has a short half life. A source of NO may produce an NO concentration higher than the sink which consumes it. Nitric oxide is toxic at high levels, and any source of nitric oxide must be regulated, either in time, by feedback, or in space. If basal NO concentration is regulated by feedback, inhibition of some sources may cause other sources to be up-regulated. The observation that autistic children have higher levels of NO metabolites may also be explained by not enough NO in the right place, so more NO is produced to compensate.

For example, the hypotension of septic shock is largely from the excess production of nitric oxide by iNOS. iNOS is the inducible form of NOS, and is an example of a "feed forward" type of control, rather than a "feed back" kind of control as in eNOS. The production of very high levels of nitric oxide by cells is best achieved by a "feed forward" type of control. Once a cell starts to produce high levels of nitric oxide, the nitric oxide so produced may inhibit the cytochrome oxidase of the mitochondria in those cells and will interfere with normal cell metabolism.

G. Stefano et al. have shown that the production of basal nitric oxide by human granulocytes has been shown to be time periodic, with a period of a few minutes, and in the 1000 pM range. George B. Stefano, et al., Cyclic nitric oxide release by human granulocytes and invertebrate ganglia and immunocytes: nano-technological enhancement of amperometric nitric oxide determination, Med Sci Monit, 2002;8(6): BR199-204 These measurements were done 10 μm above a pellet of 10E3 cells. This periodic signal was necessarily an average from many cells. That a periodic signal was observed indicates that the cells were producing NO at a time varying rate, and that this NO production was in phase. Maintaining phase coherence over so many cells would indicate communication between cells, and feedback control of NO release. It is possible that some other messenger molecule mediates the communication between cells, however any such molecule would need to have a shorter lifetime and more rapid diffusion than NO in order to maintain phase coherence. However, there may be direct sensing of nitric oxide concentration, and feedback regulation of nitric oxide production, albeit with a time lag.

Basal NO levels cannot be measured and regulated at the site of NO production because the site of NO production is necessarily above basal levels. NO must be measured remotely and the signal transmitted through a non-NO transmitter to the cells that are producing the basal NO.

An "exercise" hypothesis would argue that since nitric oxide is produced in response to physical activity, humans may have evolved to rely upon the nitric oxide produced by the moderate physical activity needed for a hunter-gatherer lifestyle. "Normal" physical activity levels may have produced sufficient nitric oxide, and so there was may have been no evolutionary pressure to evolve other nitric oxide sources. However, prehistoric infants and toddlers were not hunter gatherers. Their food was hunted and gathered by their caretakers who may well have been more physically active than modern caretakers. The physical activity level of pre-crawling or pre-walking children may not have been much higher in prehistoric times. However, an unrecognized source of nitric oxide upon which humans relied during prehistory may be that of the commensal autotrophic ammonia oxidizing bacteria, and that the frequent bathing of a modern lifestyle removes this source of nitric oxide.

Autotrophic ammonia oxidizing bacteria as a source of NO:

Commensal autotrophic ammonia oxidizing bacteria present on the skin and in particular on the scalp to generate physiologic NO from the urea in sweat, provides a rational for sweat excretion other than as a cooling mechanism. Adrenergic sweating occurs during stimulation of the adrenergic system. Adrenergic sweating occurs during periods of stress and also commonly occurs at night. It may be that that sweating on the scalp at night may serve to administer a fairly high dose of NO to the brain and to thereby "reset" the NO signaling pathways and allow the brain to do all the "housekeeping" functions that require high NO levels.

These bacteria have not been identified as associated with the human body because they do not cause any disease. In fact, they likely cannot cause disease (probably not even in immunocompromised individuals). Autotrophic ammonia oxidizing bacteria (AAOB) are obligate autotrophic bacteria as noted by Alan B. Hooper and A. Krummel at al. Alan B. Hooper, Biochemical Basis of Obligate Autotrophy in Nitrosomonas europaea, Journal of Bacteriology, Feb 1969, p. 776-779. Antje Krummel et al., Effect of Organic Matter on Growth and Cell Yield of Ammonia-Oxidizing Bacteria, Arch Microbiol (1982) 133: 50-54. AAOB derive all

metabolic energy only from the oxidation of ammonia to nitrite with nitric oxide (NO) as an intermediate product in their respiration chain and derive virtually all carbon by fixing carbon dioxide. AAOB are incapable of utilizing carbon sources other than a few simple molecules. P Chain et al. have reported a complete genome of one of them (Nitrosomonas europaea) which has been sequenced and it has only 2460 genes that code for proteins. Patrick Chain et al., Complete Genome Sequence of the Ammonia-Oxidizing Bacterium and Obligate Chemolithoautotroph Nitrosomonas europaea. Journal of Bacteriology, May 2003, p. 2759–2773. From an inspection of the genome, it is clear that these bacteria cannot cause disease. There are no genes for toxins or lytic enzymes. They do not have the metabolic machinery to utilize the complex organic compounds such as are found in animal tissues. They do not grow on any heterotrophic media such as is used for isolating pathogens (all of which are heterotrophic as reported by M Schaechter). Moselio Schaechter, Gerald Mendoff, David Schlessinger, ed., Mechanisms of Microbial Disease, Williams & Wilkins, Baltimore, MD, USA, 1989.

It may be that AAOB have not been found on the human body is that no one has looked for them with the proper culture media and techniques. They are also slow growing with optimum doubling times of 10 hours compared to 20 minutes for heterotrophs. Attempted isolation on media suitable for heterotrophs would result in overgrowth by heterotrophs because of the 30-fold faster doubling rate. They are universally present in all soils where they are responsible for the first step in the oxidation of ammonia into nitrate in the process of nitrification. As autotrophic bacteria, they are incapable of growing anywhere that lacks the substrates they require, ammonia or urea, oxygen, mineral salts. These substrates may be abundantly available on the unwashed skin from sweat residues, and in the "wild" and in the absence of frequent bathing with soap, humans would be unable to prevent the colonization of their external skin with these bacteria. These bacteria may be beneficial and commensal, and that many aspects of human physiology may have evolved to facilitate the growth of these bacteria and the utilization of the NO they so abundantly produce.

Another factor that perhaps has prevented their isolation may be the bathing practices in developed regions. It has become customary to bath with sufficient frequency so as to prevent the development of body odor. Body odor generally occurs after a few days of not bathing, and the odor compounds are generated by

heterotrophic bacteria on the external skin which metabolize exfoliated skin and sweat residues into odiferous compounds. In 3 days, autotrophic bacteria could double approximately 7 times for approximately a 100-fold increase over the post bathing population. In contrast, heterotrophic bacteria could double approximately 200 times for a 10e+60-fold increase. Heterotrophic bacterial growth would be nutrient limited. Assuming similar kinetics of removal through bathing of autotrophic and heterotrophic bacteria, controlling heterotrophic bacteria though bathing would reduce autotrophic bacteria to low, perhaps undetectable levels.

The inventor has found that a sufficient population of AAOB on the skin substantially suppresses body odor due to heterotrophic bacteria. The inventor has applied AAOB to his skin and has refrained from bathing for 2 years now, including two summers. There is essentially no body odor associated with sweating. In fact, sweating decreases body odor by nourishing the AAOB and enhancing their production of NO and nitrite. During the winter, with decreased sweating due to low ambient temperatures, there was an increase in odor. However, with increased clothing, (wearing sweaters) the inventor was able to increase basal sweating and reduce body odor to near zero again. There has been no itching, no rashes, no skin infections, no athlete's foot infection, and substantially no foot odor.

L Poughon et al. have reported that AAOB produce nitric oxide as an intermediate in their normal metabolism. Laurent Poughon, et al., Energy Model and Metabolic Flux Analysis for Autotrophic Nitrifiers. Biotechnol Bioeng 72: 416–433, 2001. D. Zart et al. have demonstrated one strain had optimum growth at concentrations of NO in air around 100 ppm (highest level tested in this study). Dirk Zart, et al., Significance of gaseous NO for ammonia oxidation by Nitrosomonas eutropha. Antonie van Leeuwenhoek 77: 49–55, 2000. AAOB can tolerate higher levels. I. Schmidt has shown that with other strains, there was no decline in NH3 consumption from 0 to 600 ppm (anaerobic in Ar plus CO_2) but it declined by 1/3 at 1000 ppm NO. Ingo Schmidt et al., Anaerobic Ammonia Oxidation in the Presence of Nitrogen Oxides (NOx) by Two Different Lithotrophs, Applied And Environmental Microbiology, Nov. 2002, p. 5351–5357. Most AAOB are aerobic, but some strains can utilize nitrite or nitrate in addition to oxygen which increases the NO production. 1000 ppm NO in air corresponds to about 2 μM/L in aqueous solution. The strain used by the inventor has produced a measured NO concentration of 2.2 μM/L. Most studies of AAOB metabolism have been motivated by their

utilization in waste water treatment processes for ammonia and nitrate removal from waste water. Operation of waste water treatment facilities at hundreds of ppm NO is undesirable, so it is not unexpected that the physiology of these bacteria under those conditions has not been well studied.

The inventor has noticed that a number of characteristics which may be associated with Asperger's have changed since applying these bacteria. It has become more difficult to "multi-task". Stimuli are more distracting, that is it is not as easy as it used to be to work while distracting stimuli are present. However, learning new information is easier, and that information is better integrated with previous information.

Subjectively, the sleeping pattern of the inventor has subjectively changed, in that he now awakes less frequently during the night. The inventor's senses of smell and touch have subjectively become more acute, and threshold stress for joint pain has seemingly decreased. These changes while subjective are consistent with increased NO levels. The inventor and others have noticed that dreams are more vivid after application of these bacteria to the scalp demonstrating an affect of increased No on a normal neurological process.

What is claimed is:

Claims

1. A method of treating autism spectrum symptoms in a subject comprising:

identifying a subject who has developed or is at risk of developing an autism spectrum symptom; and

positioning ammonia oxidizing bacteria in close proximity to the subject.

2. The method of claim 1, wherein the act of positioning ammonia oxidizing bacteria comprises:

applying ammonia oxidizing bacteria to a surface of the subject in an effective amount to cause the bacteria to metabolize any of ammonia, ammonium salts, or urea on the surface into any of nitric oxide, nitric oxide precursors or combinations thereof.

3. The method of claim 2, wherein the act of applying the bacteria comprises applying the bacteria in a suitable carrier.

4. The method of claim 2, wherein the act of applying the bacteria comprises applying a bacteria selected from the group consisting of any of Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocystis, Nitrosolobus, Nitrosovibrio, and combinations thereof.

5. The method of claim 2, wherein the act of applying the bacteria to a surface comprises applying the bacteria to skin, hair, or a combination thereof.

6. The method of claim 2, wherein the act of applying the bacteria comprises applying a substantially pure bacteria.

7. The method of claim 2, wherein the act of applying the bacteria comprises:

applying the bacteria to an article; and

contacting the article with the surface of the subject.

8. The method of claim 2, wherein the act of applying the bacteria comprises applying the bacteria mixed with an acid.

9. The method of claim 2, wherein the act of applying further comprises metabolizing any of urine, feces, blood, wound secretions, menstrual secretions, vaginal secretions, topically applied mixtures, and combinations thereof.

10 The method of claim 2, further comprising the act of applying a compound selected from any of a component of perspiration, urea, nitrite, lactic acid, nitrate, salt, iron salts, ammonium salts, and combinations thereof, to the surface of the subject .

11. The method of claim 2, further comprising:

applying at least one of urea or metal salts to the surface of the subject in an effective amount to stimulate the growth of the bacteria.

12. The method of claim 5, wherein the act of applying the bacteria to a surface of a subject includes applying the bacteria to the skin, hair, or a combination thereof, of a human.

13. The method of claim 5, wherein the act of applying the bacteria comprises applying the bacteria to the subject wherein the subject has an Autism Spectrum Disorder.

14. The method of claim 5 where the act of applying the bacteria comprises applying the bacteria to the subject wherein the subject is at risk for acquiring one or more of the characteristics of autism spectrum symptoms.

15. The method of claim 7, wherein the act of contacting the article with the surface of the subject further comprises contacting the bacteria with the surface of the subject.

16. The method of claim 7, wherein the act of contacting the article with the surface of the subject further comprises contacting a fluid excretion from the surface of the subject with the bacteria.

17. The method of claim 7, wherein the act of applying the bacteria comprises applying the bacteria to an article of clothing.

18. The method of claim 7, wherein the act of applying the bacteria mixed with an acid comprises applying the bacteria mixed with lactic acid.

19. The method of claim 10, wherein the acts of applying the compound and the bacteria include applying compound and the bacteria in a mixture.

20. The method of claim 13, wherein the act of applying the bacteria comprises applying the bacteria to an underarm surface.

21. The method of claim 17, wherein the act of applying the bacteria to the article of clothing comprises the acts of:

wearing the article and impregnating the article with the bacteria.

22. The method of claim 17, wherein the act of applying the bacteria to the article of clothing comprises the acts of: wearing the article and coating the article with the bacteria.

23. The method of claim 17, wherein act of applying the bacteria comprises applying the bacteria to a diaper.

24. The method of claim 17, wherein the act of applying the bacteria comprises applying the bacteria to an article of clothing selected from the any of shoe, sneaker, belt, hat, undergarment, pajama, athletic garment, sock, shoe insert, bandage, face mask, scarf, tampon, and condom.

25. The method of claim 21, wherein the act of impregnating the article includes contacting the article with a culture media containing the bacteria.

26. The method of claim 25, wherein the act of contacting the article with a culture media containing the bacteria comprises applying an animal manure.

27. The method of claim 25, wherein the act of contacting the article with a culture of media containing the bacteria comprises contacting the article with a substantially pure culture of bacteria.

28. The method of claim 2, wherein the act of positioning the bacteria comprises:

applying the ammonia oxidizing bacteria the a surface of the subject in an amount effective to metabolize a normal bodily secretion on the surface into any of nitric oxide, nitric oxide precursors, or combinations thereof.

29. The method of claim 28, wherein the act of applying bacteria comprises applying the bacteria to the skin between intervals of bathing.

30. The method of claim 28, wherein the act of metabolizing a normal constituent of skin secretion comprises metabolizing a component of perspiration.

31. The method of claim 29, wherein the act of applying the bacteria between intervals of bathing includes applying the bacteria at an interval of at least about one day.

32. The method of claim 30, wherein the act of metabolizing comprises metabolizing a component of perspiration selected from any of urea, nitrite, lactic acid, nitrate, salt, iron salts, ammonium salts, and combinations thereof.

33. The method of claim 31, wherein the act of applying the bacteria between intervals of bathing comprises applying the bacteria at an interval that is greater than or equal to about one week.

34. The method of claim 29, wherein the at of applying the bacteria is applied to a subject who lacks bathing facilities, is immobile, or has a bandage.

35. The method of claim 2, further comprising applying any of urea, ammonium salts, sodium, potassium, magnesium, calcium, phosphate, chloride, sulfate, trace

mineral salts, iron, copper, zinc, cobalt, manganese, molybdenum, buffers, and combinations thereof to the surface of the subject in an effective amount to stimulate the growth of the bacteria.

36. A preparation to be applied to a surface of a subject to an treat autism spectrum symptom of the subject comprising ammonia oxidizing bacteria that metabolizes perspiration when present, into any of nitric oxide, nitric oxide precursors, or combinations thereof.

37. The preparation of claim 36, wherein the preparation is any of a cosmetic composition, a body deodorant, or an athletic preparation.

38. The preparation of claim 36, wherein the bacteria is selected from any of Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocystis, Nitrosolobus, Nitrosovibrio, and combinations thereof.

39. The preparation of claim 36, wherein the component of perspiration is selected from any of urea, nitrite, lactic acid, nitrate, salt, iron salts, ammonium salts, and combinations thereof.

40. The preparation of claim 37, further comprising at least one component selected from any of water, mineral oil, coloring agent, perfume, aloe, glycerin, sodium chloride, pH buffers, UV absorbing agents, silicone oil, natural oil, vitamin E, herbal concentrates, Lactic acid, citric acid, talc, clay, calcium carbonate, magnesium carbonate, zinc oxide, starch, urea, nitrite, nitrate, iron salts, ammonium salts, and combinations thereof.

41. The preparation of claim 37, wherein the preparation is any of powder, cream, stick, aerosol, or salve.

42. The preparation of claim 36, wherein the surface of the subject is of a human being.

43. The preparation of claim 36, further comprising a component selected from any of water, mineral oil, coloring agent, perfume, aloe, glycerin, sodium chloride, pH buffers, UV absorbing agents, silicone oil, natural oils, vitamin E, herbal concentrates, lactic acid, citric acid, talc, clay, calcium carbonate, magnesium carbonate, zinc oxide, starch, urea, nitrite, lactic acid, nitrate, iron salts, ammonium salts, and combinations thereof.

44. The preparation of claim 36, further comprising:

at least one compound selected from any of urea, ammonium salts, sodium, potassium, magnesium, calcium, phosphate, chloride, sulfate, trace mineral salts, iron, copper, zinc, cobalt, manganese, molybdenum, buffers, and combinations thereof.

45. An article of clothing used to treat autism spectrum symptoms of a subject comprising:

the article of clothing treated with bacteria adapted to metabolize any of ammonia, ammonium salts, or urea into any of nitric oxide, nitric oxide precursors, or combinations thereof.

46. The article of claim 45, wherein the ammonia, ammonium salts, or urea are a component of any of perspiration, urine, feces, blood, wound secretions, menstrual secretions, vaginal secretions, topically applied mixtures, and combinations thereof.

47. The article of claim 45, wherein the article of clothing is a hat.

48. The article of claim 45, wherein the article is selected from any of a hat and scarf.

49. The article of claim 45, wherein the bacteria is an ammonia oxidizing bacteria.

50. The article of claim 45, further comprising at least one of urea, nitrite, lactic acid, nitrate, salt, iron salts, ammonium salts, and combinations thereof.

51. The article of claim 49, wherein the bacteria is selected from any of Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocystis, Nitrosolobus, Nitrosovibrio, and combinations thereof.

Abstract

A method of treating autism spectrum symptoms through the generation in close proximity of a surface of the subject, nitric oxide and nitric oxide precursors using bacteria adapted to oxidize ammonia and urea derived from perspiration is described.

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